REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ORGANIZATION.

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
30-06-2008	Final Report	1 Sept 2007 to 30 June 2008

4. TITLE AND SUBTITLE
"(STTR PHI) Photochemical Tissue Bonding for Military Medical Applications"

5a. CONTRACT NUMBER
FA9550-07-C-0098

5b. GRANT NUMBER
N/A

5c. PROGRAM ELEMENT NUMBER
N/A

6. AUTHOR(S)
Dennis McCal - nLIGHT Photonics
Scott Prahl - OMLC

5d. PROJECT NUMBER
N/A

5e. TASK NUMBER
N/A

5f. WORK UNIT NUMBER
N/A

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
nLIGHT PHOTONICS CORPORATION
5408 NE 88ST STE E
VANCOUVER, WA 98665-0990

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
USAF, AFRL DUNS 143574726
AF OFFICE OF SCIENTIFIC RESEARCH
875 N. RANDOLPH ST, ROOM 3112
ARLINGTON VA 22203

10. SPONSOR/MONITOR'S ACRONYM(S)
AFRL

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited.

13. SUPPLEMENTARY NOTES
N/A

14. ABSTRACT
Report developed under STTR contract for topic number AF07-T033. The overall goal is to develop a complete system for micro-anastomosis of blood vessels. This involves (1) a unique laser system that uses water as the absorbing chromophore, (2) a clinically useful handpiece that is appropriate for microsurgery, (3) a novel albumin stent to support the vessel during anastomosis, (4) in vitro testing of the device to assess thermal damage, strength, and operative time. And (5) In Vivo animal testing will be added to Phase II. The goals of providing a 1.9um laser and an appropriate handpiece were accomplished in Phase I. Similarly the goals of manufacturing prototype albumin stents and applying them in vitro testing were met and proven effective by collection of pull test data to access joint strength, thermal damage studies and dissolution studies following application of the stents and laser "soldering" process to repair swine blood vessels. A Phase II program, if funded, is expected to lead to development of a commercially testable system for micro-anastomosis of blood vessels.

15. SUBJECT TERMS
micro-anastomosis, albumin stent, 1.9 micron laser diodes, STTR Report

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Dennis McCal
U	U	U	SAR		19b. TELEPHONE NUMBER *(Include area code)* 503-214-5331

(STTR PH1) Photochemical Tissue Bonding for Military Medical Applications

STTR AF07-T033

Dennis M. McCal (nLIGHT), Scott A Prahl (Oregon Medical Laser Center)

nLIGHT PHOTONICS CORPORATION
5408 NE 88ST STE E
VANCOUVER, WA 98665-0990

June 2008
Final Report

Project period from 1 Sept 2007 to 30 June 2008

USAF, AFRL DUNS 143574726
AF OFFICE OF SCIENTIFIC RESEARCH
875 N. RANDOLPH ST, ROOM 3112
ARLINGTON VA 22203

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ORGANIZATION.

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
30-06-2008	Final Report	1 Sept 2007 to 30 June 2008

4. TITLE AND SUBTITLE
"(STTR PHI) Photochemical Tissue Bonding for Military Medical Applications"

5a. CONTRACT NUMBER
FA9550-07-C-0098

5b. GRANT NUMBER
N/A

5c. PROGRAM ELEMENT NUMBER
N/A

6. AUTHOR(S)
Dennis McCal - nLIGHT Photonics
Scott Prahl - OMLC

5d. PROJECT NUMBER
N/A

5e. TASK NUMBER
N/A

5f. WORK UNIT NUMBER
N/A

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
nLIGHT PHOTONICS CORPORATION
5408 NE 88ST STE E
VANCOUVER, WA 98665-0990

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
USAF, AFRL DUNS 143574726
AF OFFICE OF SCIENTIFIC RESEARCH
875 N. RANDOLPH ST, ROOM 3112
ARLINGTON VA 22203

10. SPONSOR/MONITOR'S ACRONYM(S)
AFRL

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited.

13. SUPPLEMENTARY NOTES
N/A

14. ABSTRACT
Report developed under STTR contract for topic number AF07-T033. The overall goal is to develop a complete system for micro-anastomosis of blood vessels. This involves (1) a unique laser system that uses water as the absorbing chromophore, (2) a clinically useful handpiece that is appropriate for microsurgery, (3) a novel albumin stent to support the vessel during anastomosis, (4) in vitro testing of the device to assess thermal damage, strength, and operative time. And (5) In Vivo animal testing will be added to Phase II. The goals of providing a 1.9um laser and an appropriate handpiece were accomplished in Phase I. Similarly the goals of manufacturing prototype albumin stents and applying them in vitro testing were met and proven effective by collection of pull test data to access joint strength, thermal damage studies and dissolution studies following application of the stents and laser "soldering" process to repair swine blood vessels. A Phase II program, if funded, is expected to lead to development of a commercially testable system for micro-anastomosis of blood vessels.

15. SUBJECT TERMS
micro-anastomosis, albumin stent, 1.9 micron laser diodes, STTR Report

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Dennis McCal
U	U	U	SAR		19b. TELEPHONE NUMBER (Include area code) 503-214-5331

Reset

Table of Contents

Table of Figures

1. Summary

Joining severed vessels is a recurring problem in trauma and surgery. The basic technology of joining (or anastomosing) vessels using sutures has been available for centuries, but remains a slow and tedious process. Many technologies have been introduced to make vessel suturing water-tight. Any solution to this problem must integrate well with standard medical care. This means that the solution must be safe, effective, acceptable to surgeons, and technologically feasible.

The overall goal of this work was to develop a complete system for micro-anastomosis of vessels. This involved (1) a unique laser system that uses water as the absorbing chromophore, (2) a clinically useful hand piece that is appropriate for microsurgery, (3) a novel albumin stent to support the vessel during anastomosis, and (4) in vitro testing of the device to assess thermal damage, strength, and operative time.

The key points of this report are that (1) a novel two-wavelength laser system (1.9 and 0.639 micron wavelengths) was produced, (2) a ergonomic handpiece was designed and built, (3) albumin stents were made in a range of sizes and (4) all three components were tested using by joining vessels on the bench top.

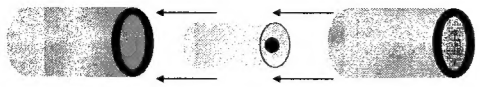
The major result of this work was that the two-wavelength system was effective at joining vessels with strong, water-tight junctions. We found that the albumin stents were useful in the anastomosis process, but it was necessary to closely match the diameter of the stent to that of the vessel; failure to do so made the repair process slower. We also found that the red aiming beam (0.639 micron) created a diffuse spot which inhibited the ability of the surgeon to fully exploit the microsurgical precision of the 200 micron spot that the laser produced on the surface of the vessel. Finally, we found that the hand piece design was critical to success of the laser welding process.

We conclude that the two-wavelength system shows exciting promise. We recommend that the aiming beam be changed to a shorter wavelength (green instead of red). We further recommend that albumin stents be produced (under GLP conditions) using molds in a range of sizes from 3-7mm in diameter at 0.5mm increments. With these refinements, the two wavelength anastomosis system can be tested using in vivo pre-clinical experiments.

2. Introduction

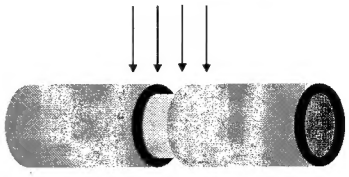
This report is focused on the application of 1.9um light to successful laser-assisted vascular anastomosis rather than the nature of the laser die fabrication process that makes it possible. To that end our introduction concentrates on the use of the laser in that application. Descriptions of the laser are provided in section 3.

We proposed developing a novel two-wavelength laser that allows precise and rapid closure of anastomosis of vessels – illustrated schematically in Figure 1. An exogenous glue, composed entirely of FDA-approved human serum albumin is used to increase bond strength and burst pressures. A dissolvable stent, also composed of human serum albumin, is used to facilitate joining larger vessels and aligning the vessel edges.



Albumin provides (1) support, (2) glue for the laser bond

1.9 micron laser light



Any non-irradiated albumin will dissolve as soon as blood flow starts

Figure 1 Albumin Stents

Illustration of the technique Left: An albumin stent is inserted into a broken blood vessel. Liquid albumin is also applied on the outer surface as a "glue". Right: The stent and albumin glue are illuminated with 1.9 micron light, which heats the albumin, "curing" the glue to form a robust bond.

Since the first reported [1] successful laser-welded vascular anastomosis in 1979, this method has held significant promise but never become popular. Laser welding has remarkable advantages over traditional suture such as providing an immediate watertight sealant [2], reducing operative time [3, 4, 5], faster healing [6], and reduced intimal hyperplasia owing to no foreign body reaction to suture material [7]. However, the main disadvantages of the laser-assisted procedure are the low strength of the resulting anastomosis [2], especially in the acute healing phase up to 4 days post-operatively [8], and increased anastomotic pseudoaneurysm rate [3, 5, 6, 7]. From a surgical viewpoint, a satisfactory requirement of laser tissue bonding is to obtain maximum bond strength with minimal tissue thermal injury.

It has been shown that laser tissue bonding using diode laser and albumin solder with indocyanine green (ICG) is an effective technique in surgical reconstruction such as in blood vessels [17, 18, 19] urinary tract [20], and skin [21, 22]. It is known that the albumin solder with higher concentration resulted in significantly stronger tensile strength than the albumin solder with lower concentration [23, 24]. Poppas has used highly concentrated albumin solders to improve laser bond strengths [25] and others have used solid albumin [24]. Finally, our group and others have used pulsed lasers to further reduce collateral thermal damage during laser bonding [26].

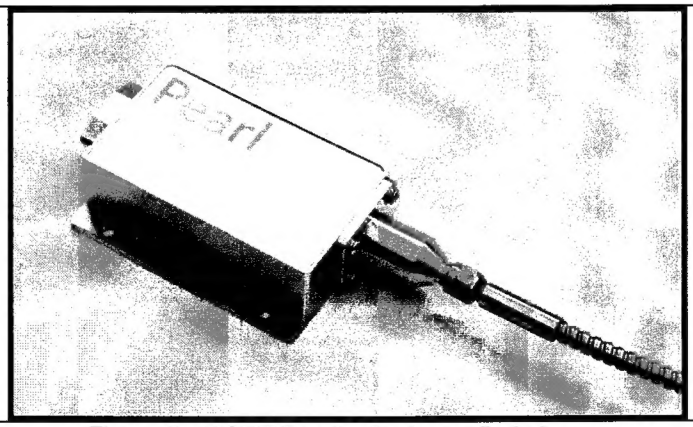


Figure 2 nLIGHT Pearl 1.9 micron diode lasers

nLIGHT Laser innovations have lead to the availability of high performance 1.9 micron diode laser sources. These lasers are integrated in nLIGHT's fibered module that delivers the light from a small core fiber suitable for medical use.

Despite these innovations, laser bonding has not gained widespread clinical acceptance because (1) an exogenous chromophore (indocyanine green) was required, (2) no economical laser sources, (3) large beam sizes (1mm or greater), (4) no internal support for the vessel during anastomosis was available. Recent innovations in laser technology at nLIGHT Corp remove issues (2) and (3). nLIGHT now provide high performance 1.9 micron diode laser sources at prices consistent with widespread medical use – an example is shown in Figure 2. The protocol proposed here would eliminate issues (1) and (4) – water replaces indocyanine green as the light absorbing chromophore, and internal support is provided by a solid albumin stent, which itself dissolves once blood is allowed to flow again.

Other groups have used photochemical tissue bonding as a technique for sealing tissue by chemical cross-linking. Generally only two photochemically active dye have been used: Rose Bengal (a xanthene dye) and naphthalimides. Typically the light causes the dye to produce reactive species that interact with potential electron donors and acceptors (e.g., tryptophan, tyrosine) of proteins to produce covalent bonds. No work has been published on the naphthalimides for the last decade [9, 10] and no long term cell viability studies have been published. Rose Bengal has been used successfully in acellular tissues [11, 12, 13, 14, 15], but recently was found to be incompatible with chrondrocyte cell growth [16]. This illustrates a fundamental problem with photochemical processes: if the dye becomes photoactive, it can cause covalent cross-linking within cells. Furthermore, any residual reactive species that have not interacted are potential foci for later problems. This means getting FDA approval will be difficult for anything (except potentially external applications).

Photothermal bonding is attractive because no highly reactive chemical species are involved. We use human serum albumin as a protein to fill the voids in the anastomosis. Furthermore, if only albumin is used then thermal coagulation is safe. We know this because there are thousands of cases each day of thermal coagulation of albumin during electrocautery. Albumin is present at a concentration of about six percent in whole blood. Since electrocautery is used to stop bleeding, albumin must be coagulated every time an electrocautery device is used.

To deliver the light to a tissue, the light must be absorbed by naturally present chromophores – specifically water. There are two attractive features to 1.9 micron emission: (1) there is an absorption peak meaning only low power levels are needed, (2) the penetration depth is 100-micron – sufficient to generate a physically robust bond, but small enough that the light cannot lead to significant damage of healthy tissue underneath, Figure 3. These factors combine to make 1.9 micron an ideal wavelength for laser operation.

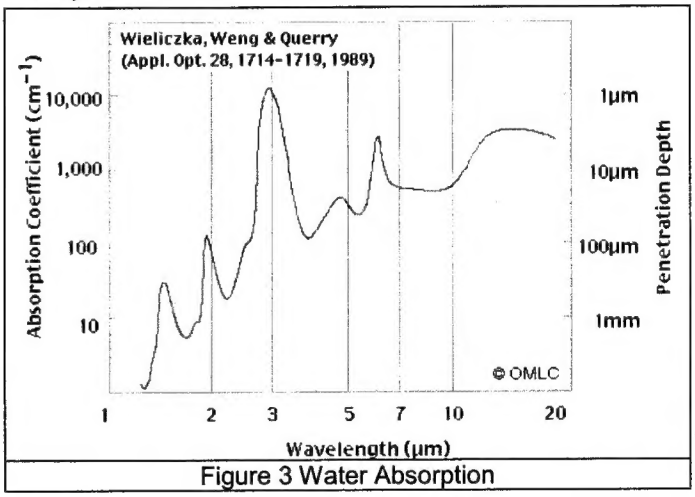


Figure 3 Water Absorption

For surgical applications, a visible guide beam must also be present. Thus in principle, a compact two laser diode device (1.9 microns and 0.54 microns) in a small laser pointer form factor (like a pen) that formed a 0.1mm laser spot 5-10 mm from the front face of the pen would give surgeons precise, controllable method for laser welding. The fibered laser unit shown in Figure 2 allows nLIGHT to deliver multiple laser wavelengths into a single fiber and allowed the medical procedure to be developed through Phase 1, with a visible red (0.639 micron) source combined with 1.9 micron light in a fibered source as shown in Figure 2. In later phases red would be replaced by visible green (0.54 micron) in a simpler lower-cost rugged sterlizable unit suitable for practical surgical use.

3. Methods, Assumptions and Procedures

3.1. Aim 1: Two wavelength laser system

In Phase I, nLIGHT promised to "Develop a dual wavelength laser system that delivers 300mW of 1.9 micron light and 1 mW of 0.639 micron light through a 100 micron fiber." During the course of Phase I, a laser system was provided that was capable of providing up to 1W of laser light at 1.9 micron and more than sufficient red laser light to indicate the beam location. nLIGHT provided the light via a 200 micron fiber and modified the hand piece optics to accommodate the difference from the original plan.

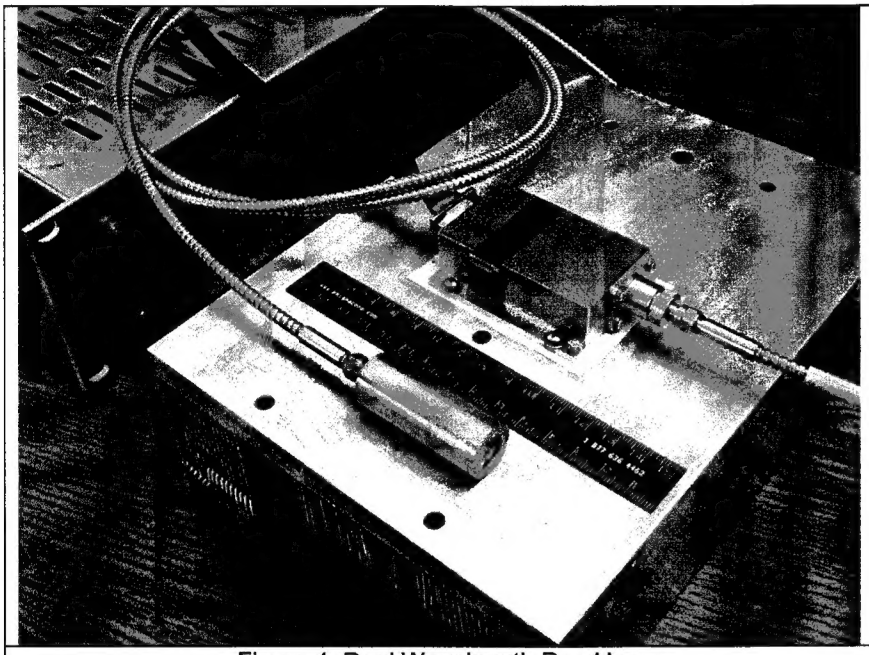


Figure 4: Dual Wavelength Pearl laser
Picture shows scale and the 1st hand piece design.

The laser used was based on a laser series called Pearl. This product spatially combines light from multiple AuSn-bonded, facet-passivated, high-efficiency single emitter laser diodes. The light is directed into a fiberoptic cable. There are various different fiber core sizes available. This package is capable of being populated with as many as 10 laser diodes with wavelengths ranging anywhere between 0.630 to 2.05 microns and coupled into a 100, 200, or 400 micron core diameter fiber optic fibers. Each single emitter within the package has its own fast and slow axis collimation using micro-optics to condition the laser light prior to fiber coupling. Fiber coupling is accomplished with a single bulk optic, which focuses all of the individual collimated beams into a single fiber core. In this case the fiber has a 200 micron core with an NA of 0.22. The fiber assembly is armor jacketed as shown. It uses industry standard SMA-905 connectors at both the proximal and distal ends as shown in Figure 4. In this case lasers of two different wavelength are easily handled by the Pearl architecture.

Rather than using a TE cooler the laser was mounted on a large heat sink. At 0.5W light output the 1.9um laser dissipates about 9W easily dissipated by the heat sink sufficient for the application. The broad 1.9 micron water absorption peak shown in Figure 3 allows a relatively relaxed temperature control of the laser. It was found that the change of the laser wavelength with the < 5 degrees package temperature rise was not an issue.

The drivers used to control the laser were obtained off the shelf from VueMetrix, they are in the aluminum box behind the heat sink in Figure 4. The red laser was controlled by a Vue-DPSS-3.0 and the 1.9 micron laser was controlled by a Vue-MV12-01. These laser drivers are controlled by an MS Windows based Graphical User Interface (GUI) as shown in the examples of these devices in Figure 5. The two drivers each had their own GUI that was opened to control the lasers inside the Pearl.

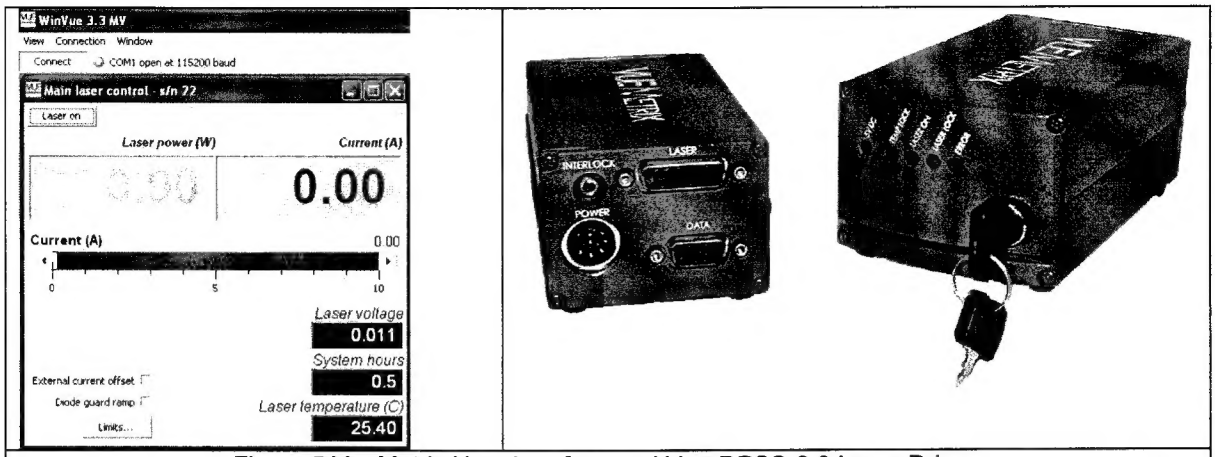


Figure 5 VueMetrix User Interface and Vue-DPSS-3.0 Laser Driver.

3.2. Aim 2: Ergonomic Handpiece

The second portion of laser application development for nLIGHT was to provide a hand piece. The hand piece was thought to require delivery of 100 micron spot at 5-10mm away from the end of the hand piece. nLIGHT delivered a hand piece with that prescription as shown in Figure 4 above. This hand piece was found through experimental use to not be a very useful tool. The diameter of the tool was too large and the focal point was too close to the hand piece so that it obscured the view of the surgeon from the work area.

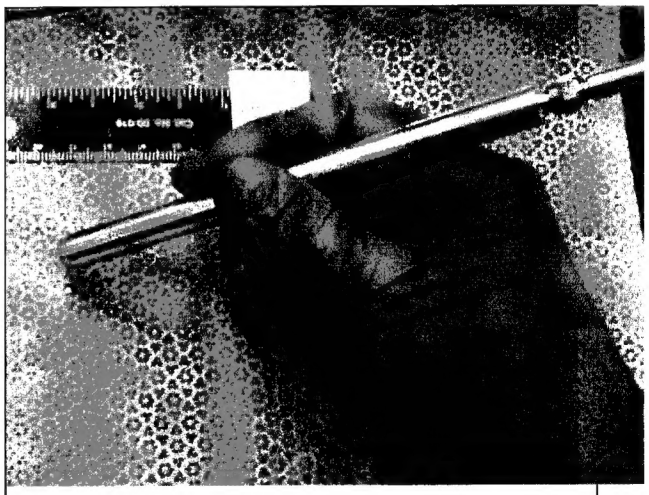


Figure 6: Second Generation Hand Piece

To alleviate that problem, nLIGHT provided a second hand piece designed for dual use (external use and laparoscopic use). The second hand piece, shown in Figure 6, had roughly the physical dimensions of a pencil: it was 150mm long and less than 10mm in diameter. This set of dimensions will allow it to be used in laparoscopic surgery. The second hand piece had a slight taper in surrounding the front lens. It was also designed to focus at 20mm from the hand piece and to have a 0.2mm minimum spot size. Both have proven to be good choices by experimental testing.

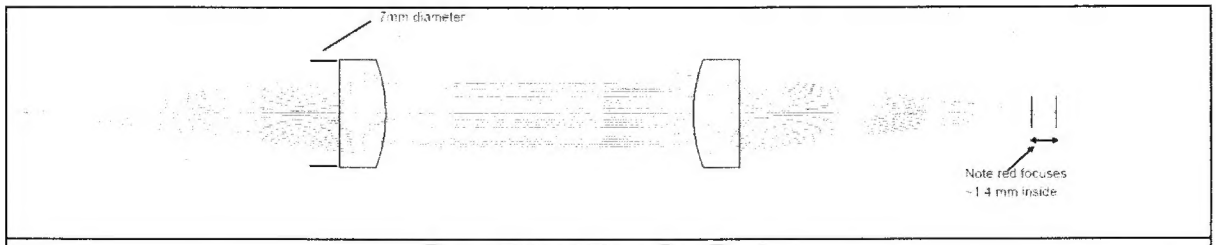


Figure 7 Hand Piece Ray Tracing

The ray tracing shows the focal point for the 1.9um laser and indicates that the red light focuses closer to the hand piece, the 1.4mm difference in focal point did not limit the surgeons use of the tool.

The difference in focal point for the two different wavelengths was calculated prior to manufacture of the hand piece and discussed with the surgeons who would be performing the procedures. They noted that the tool is applied held in free space, standing up; 1.4mm is sufficient indication of the focal point of the IR beam. It is possible to bring the two focal points closer to the same point but would have required optical coatings to be selected and required additional time and money. This will be an area for more investigation in a Phase II program. The ray trace in Figure 7 shows this effect.

3.3. Aim 3: Albumin Stent Production

Preparation of Concentrated 38% Albumin
We have implemented a new, simpler, and faster method for concentrating albumin from 25% (available from the pharmacy) to 38% (optimal for laser welding). A significant benefit of the new method is that it dramatically facilitates the production of sterile albumin. The previous method used an Amicon chamber with a 25,000 MW filter. The chamber was pressurized to 60 psi and heated to 60 degrees centigrade. Proper filtration required that the stirring bar in the chamber continue to spin as the concentration of albumin increased.

The new method uses dialysis tubing to eliminate excess water. The 25% albumin is placed in the tubing and sealed. The tubing is hung in a hood for several hours in a controlled environment. Water evaporates through the dialysis tubing walls. The concentration process is finished when the weight of the tubing and albumin has decreased by the amount of water that needed to be removed.

The dialysis tube (12,000-14,000 MWCO) is weighed and two clips are used to seal the albumin in the dialysis tube. One end of the tube is sealed with a clip. The sterile 25% albumin is injected into one meter of sterile dialysis tubing (16mm in diameter). The second end of the tube is sealed with another clip and weighed. The dialysis tubing filled with albumin was hung in a sterile flow hood and weighed periodically. Once the desired weight was reached, the albumin was placed in sterile 10 ml vials for storage at room temperature.

Preparation of Albumin Stents
The first method for producing albumin stents consisted of dipping a metal wire in albumin repeatedly. We used expired sterile 38% human serum albumin (prepared from 25%). A metal wire with a diameter of 1mm was dipped into solution once every 5 minutes for 90 minutes. The wire was completely dry before redipping. After 90 minutes only 100 microns of albumin was deposited on the outside of the wire, because successive dips tended to dissolve the previous layer. For this reason and because of the non-uniformity in the coating we abandoned this method of production.

The second method used highly concentrated albumin (72%) that was shaped around a 1mm wire. The 38% albumin was poured into glass beaker and dried in a 60°C oven for about 10 minutes. The albumin was removed from the oven while albumin was still malleable but no longer tacky. This albumin was then rolled onto the wire and shaped into a cylindrical stent. The stent was removed from the wire. This method was used for all stents in the dissolution study.

3.4. Aim 4 Anastomosis and InVitro Testing

Laser Anastomosis Experiments
Fresh arteries and veins were harvested from domestic swine with minimal trauma and immediately placed in sterile 0.9% saline solution at 4 degrees C. Some experiments used carotid arteries bought from a slaughterhouse and also kept in cold saline until use

Burst Pressure Tests
A perfusion system was set up for burst pressure testing. Initially normal saline was used to infuse the vessels, later whole porcine blood was used (after it was discovered that the stents dissolved much more slowly in porcine blood than in saline.) The fluid pressure was increased at a rate of 10mmHg/second until the vessel burst or began leaking.

Tensile Failure Testing
The bonded arteries were tested immediately after anastomosis for tensile strength. The vessel was pulled at a rate of 1mm/second. The breaking force was recorded using a tensile tester (MTS 858 Mini Bionix II).

Histology
The tissue samples were immediately fixed in 10% formalin solution after laser welding. The specimens were dehydrated and embedded with paraffin wax and then sliced longitudinally for H&E staining. The slides were observed with a Leica microscope (Leica DMRB, Germany). The area of thermal damage was distinguished by a color change and tissue disorganization.

Statistical Analysis
Statistical comparisons of all groups were examined using Student t-test. All data are expressed as average ± standard deviation. $p<0.05$ were considered statistically significant.

4. Results and Discussion

4.1. nLIGHT Laser Results

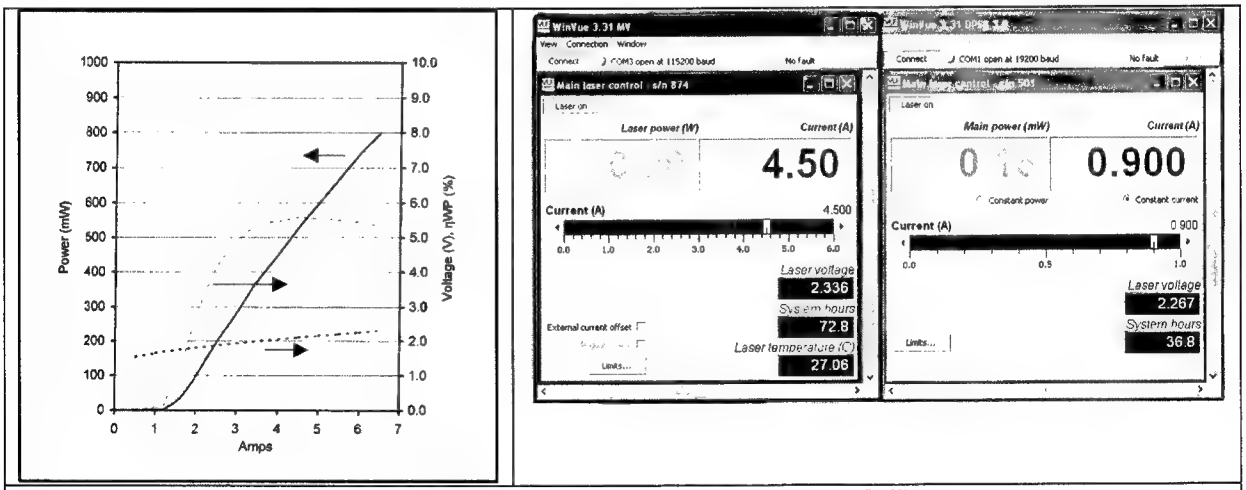


Figure 8 Laser LIV Curves and the Laser Driver GUI's

Note that the 1.9 micron laser GUI is on the left and the aiming laser GUI is on the right. Note the temperature on the IR laser GUI is provided by a thermister in the case of the Pearl.

In Phase I nLIGHT delivered a laser capable of over 500mW of 1.9 micron light, well in excess of the 300mW requirement. The actual laser used is shown in Figure 4. It was found in using the laser that 300mW was too low power and tedious for the surgeons. It was fortuitous that the initial laser provided was selected to allow over 600mW of laser light as shown in figure Figure 8

4.2. nLIGHT Handpiece Results

The operators of the system have found the red aiming beam is scattered by the tissue and consequently visual feedback to the operator is much less precise than expected. We have proposed a Phase II system which uses a green LED as an aiming beam with similar focal properties to the IR light.

It was also found that the initial hand piece provided had a diameter that was awkwardly large and tended to obscure the view of the blood vessel. To improve this a narrower hand piece, roughly 10mm diameter and 150mm in length was developed. To improve ease of use for the surgeon the working distance was moved out to 20mm from the tip of the hand piece with a 0.2mm minimum focus diameter. The size/shape of the 2nd hand piece and the working distance/minimum focal diameter for the 2nd hand piece have proven appropriate to the application.

4.3. OMLC Albumin Stent Production and Testing

One critical feature of the albumin stents is that they must dissolve. If the albumin stent is not resorbed or dissolved by the blood in the vessel, then it will block free blood flow through the vessel. We evaluated the dissolution of stents first in phosphate buffered saline and then in whole porcine blood.

Stent Dissolution in Phosphate Buffered Saline

One stent was divided into three sections and each section was immersed in 25ml of phosphate buffered saline. The sections were removed from the saline and weighed after each 60 seconds of immersion. In this experiment, the saline did not enter the inside of the stent due to surface tension. Since the outer diameter is 40% larger (1.4 vs 1.0mm), it is expected that these dissolution numbers will be about 40% higher than those that will be obtained when saline is pumped through the stents. Wetting of the stent caused an initial increase in weight that remained constant for the next four minutes. After four minutes the stent rapidly dissolved over the next minute until nothing was left after five minutes of immersion **Error! Reference source not found.**.

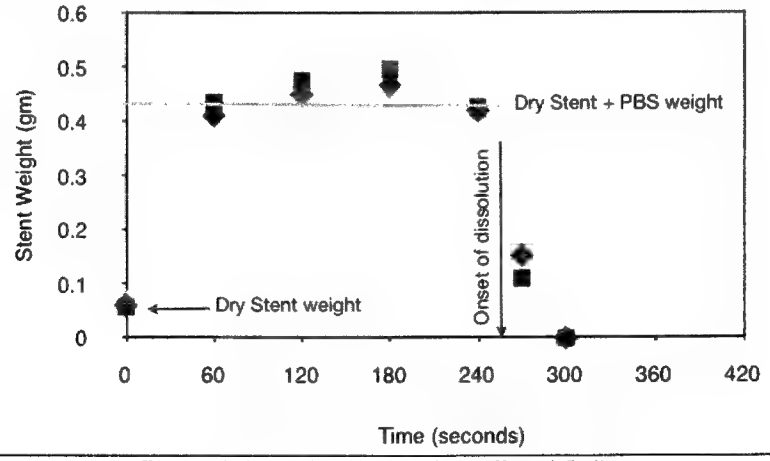


Figure 9 Stent Dissolution in Buffered Saline

Stent dissolution characteristics in phosphate buffered saline. The initial jump in stent weight was caused by wetting. The weight remained more or less constant until dissolution commenced after about 240 seconds.

Stent Dissolution in Porcine Blood

Human serum albumin was concentrated to 74% albumin and formed into four spheres. Each sphere was submerged in bovine citrated whole blood (Lampire Biologicals). The decrease in mass of the albumin

was recorded over time. The albumin was removed from the blood, rinsed with 100% ethanol, and the mass was recorded (Mettler AE200) at each time point **Error! Reference source not found.**. Despite variation in the initial mass of the spheres, the rate of dissolution was relatively constant (4.3±1.1 mg/min). The dissolution characteristics are markedly different from those of albumin in saline and must be attributed to the presence of other substances in whole blood that inhibit the dissolution process. When blood flows through albumin stents (figure 10 right), the stent is completely dissolved in about one minute. This suggests that convection is a more important for dissolution than diffusion.

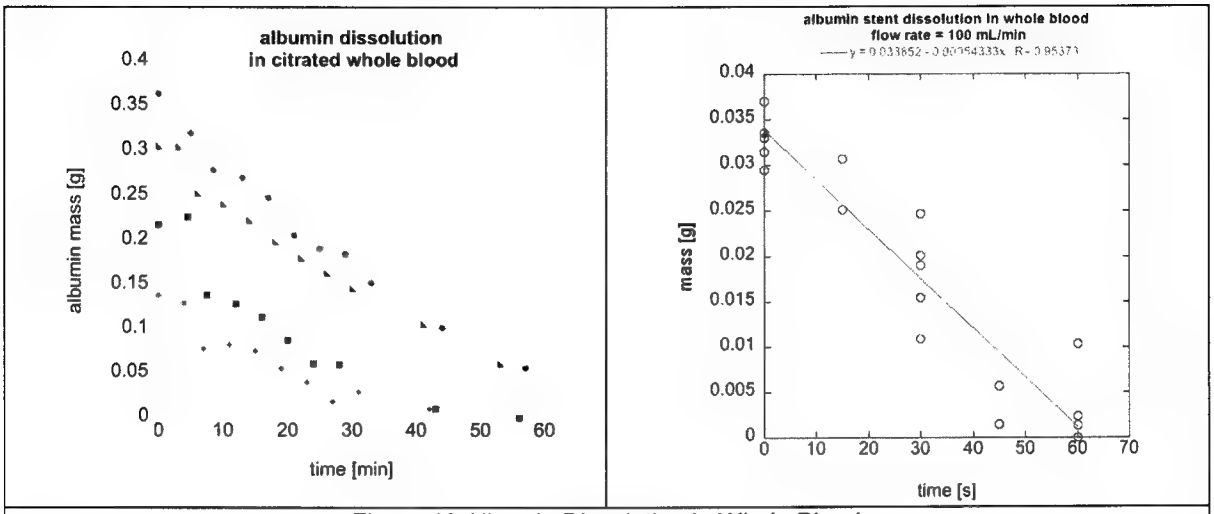


Figure 10 Albumin Dissolution in Whole Blood
Left: Dissolution curves for four albumin spheres that were immersed in whole blood at time zero. The albumin sphere was weighed as a function of time. The sphere lost 4.3±1.1 mg of albumin per minute. Right: The stents are completely dissolved in one minute when blood flows through them at 100 ml/minute.

4.4. Laser Anastomosis of Vessels

The second hand piece produced by nLIGHT had significantly better ergonomic characteristics. The smaller diameter allowed better visualization of the field by the surgeon than the initial hand piece did. The location of the focus was much more appropriate for vessel anastomosis. The vessels used were porcine carotid vessels. Two sets of repairs were made. The first set used an albumin stent and the second set did not. If a stent was used, the surgeon selected an albumin stent with the right diameter and inserted it into each end of the transected vessel. In all cases, two stay sutures (5-0 Proline) were placed to manipulate the vessel (Figure 11) and to proximate the edges. 38% albumin was then applied by the surgeon around the joint and the albumin was coagulated using the nLIGHT laser. The elapsed time for the repairs were not significantly different in the suture-only group (18±6 minutes) and in the welded group (14±5 minutes). The lack of significance was probably caused by one stent that was the wrong size and significantly delayed one laser repair.

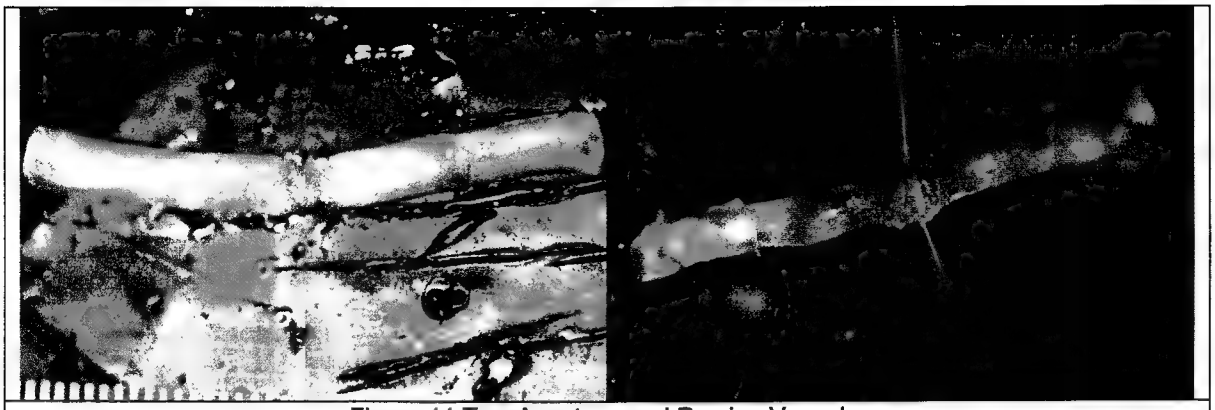


Figure 11 Two Anastomosed Porcine Vessels.

The repair on the left was made without using a stent and the one on the right used a stent. The two stay sutures visible in the right photo were cut short before the photo on the left was taken. The (defocused) red aiming beam is visible in the picture on the right.

Burst Pressure Testing of Repaired Vessels

Vessels were tied to barbed adapters with umbilical tape at each end, the other end of the adaptors was inside 2 mm ID silicone tubing. A peristaltic pump drove saline through the tubing and the vessel. The system was blocked by a valve downstream of the vessel. Between the vessel and the valve, a pressure transducer measured the pressure in the system. The pressure was recorded every millisecond. The pump was run at 10 rpm to clear all air from the line before measurements. The pump was set at 44 rpm (100 mL/min) for testing. The burst pressure was recorded for each sample (Figure 12). All vessels failed at the anastomosis site. The results showed that the albumin stent significantly increased the burst pressure (**Error! Reference source not found.**).

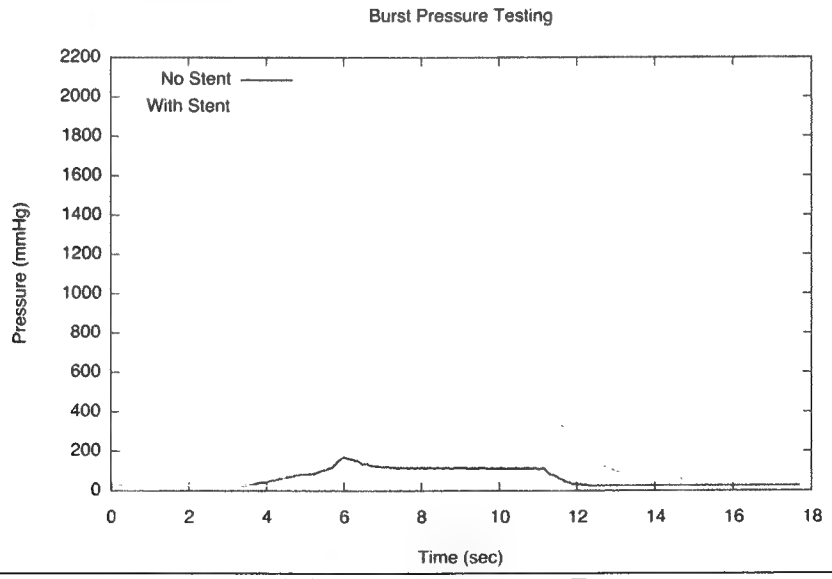


Figure 12 Typical Burst Pressure Tests.

This shows the dramatic increase in burst pressure when an albumin stent was used. In these graphs, it took about four seconds for the plastic tubing and vessel to fill with fluid. Once the tube was filled, the pressure increased rapidly until the vessel failed. In the no stent case above, the vessel leaked fluid after the six second mark.

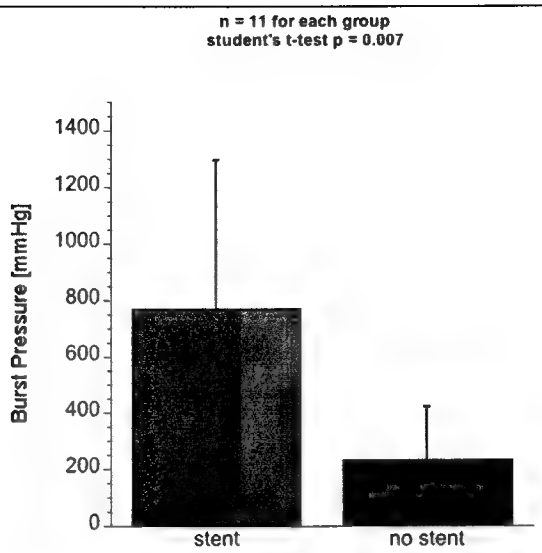


Figure 13 Burst Pressure

Burst pressures for all vessels anastomosed with and without stents. There were eleven vessels in each group and the differences between the average burst pressures were significant (p=0.007). Both stent and no-stent burst pressures exceeded normal physiological pressures by a significant amount.

Tensile Testing of Repaired Vessels

Another test for the durability of the repaired vessels is a pull or tensile test. In this test, the ends of the anastomosed vessel (about 20mm long) were clamped in a material tester (MTS 585). The tester then pulled the clamped ends of the vessel at a constant rate (1mm/s) until it failed (Figure 14). We evaluated two types of repairs: the standard suture repair and a repair using an albumin stent and coagulated 38% albumin around the vessel. Obviously the suture-only repair was much stronger than the welded vessel (Figure 15). One important clinical finding is that the vessel needed to be stretched from a resting state of 20mm to nearly 30 mm before failing. This amount of strain is unlikely to be found in a physiological setting and suggests that the laser repairs will be robust in vivo.

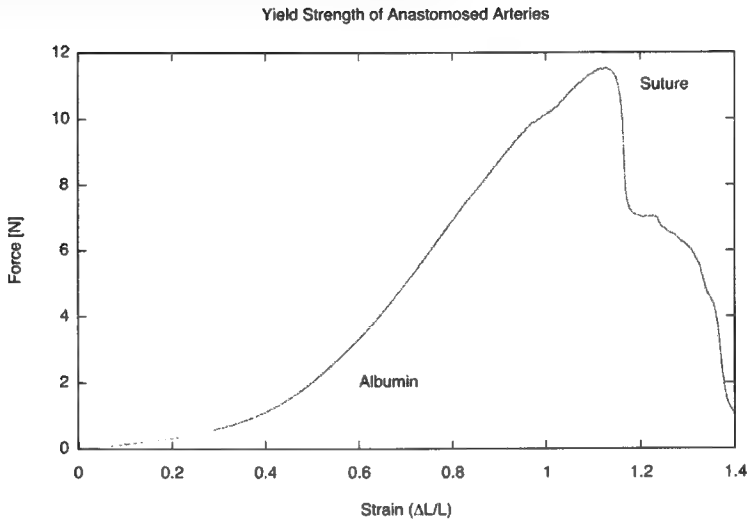


Figure 14 Tensile Testing

Typical tensile testing of vessels repaired with sutures or with an albumin stent. The two stay sutures for the albumin stent were melted using the laser before the anastomosed vessel was tested. So this graph shows the worst case scenario. Nevertheless, the albumin repair did not fail until it was pulled to more than 150% of its original length. The suture-only vessels did not pull apart at the sutures but rather at the location where the material tester held the vessel.

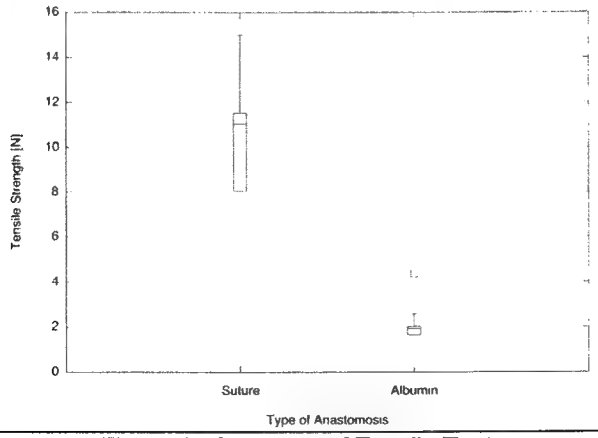


Figure 15 Summary of Tensile Tests.

The suture group was stronger (p=0.001) than the albumin group.

Histology

Our initial histology preparations used cross sections of vessels (perpendicular to the axis of the vessel). These sections were difficult to interpret and later transverse histology specimens were prepared with cuts parallel to the axis of the vessel.

In general, our observations showed that full-thickness thermal damage was produced in the vessel directly below the denatured albumin on the surface. We found that close proximation of the edges of the vessel (necessary for proper healing of the vessel) was problematic when the stents were not used (Figure 16, right). Accurate proximation of the edges tended to occur only near locations close to the stay sutures.

On the other hand, the vessels repaired with albumin stents showed excellent proximation of surfaces (figure 17). In all cases, the albumin was closely bonded to the surface of the vessel creating a durable repair. When albumin stents were used, both the inner and outer vessel surfaces were coated with albumin (figure 17 left) which creates a strong anastomotic repair (figure 18).

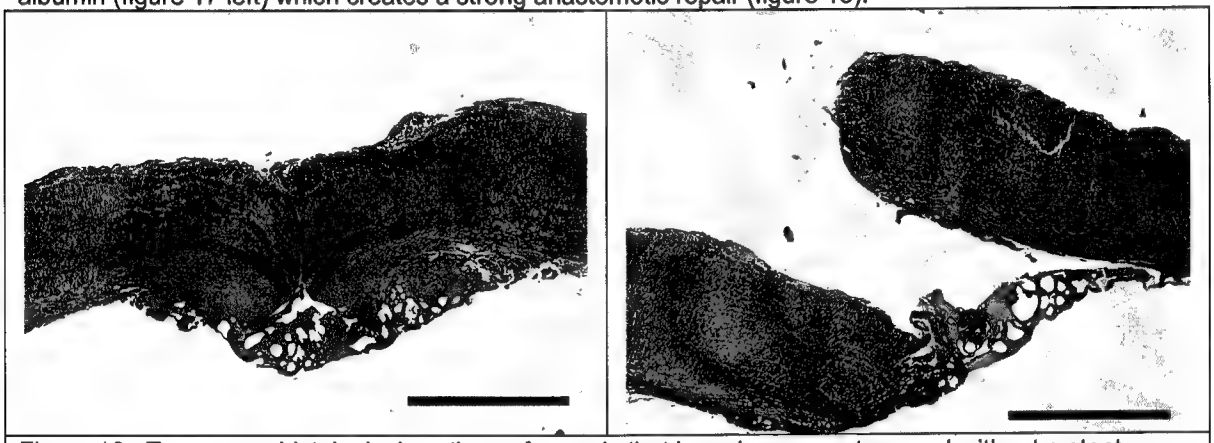


Figure 16. Transverse histological sections of vessels that have been anastomosed without a stent (black bars are 1mm in length). The inner luminal surface is up. The image on the left shows a vessel with excellent proximation of edges with the coagulated (neon pink) albumin at the bottom of the image. The image on the right illustrates that proximation of edges is an important problem; if this repair was done in vivo, the separation would be a nidus for clot formation and subject to scar formation and stenosis.

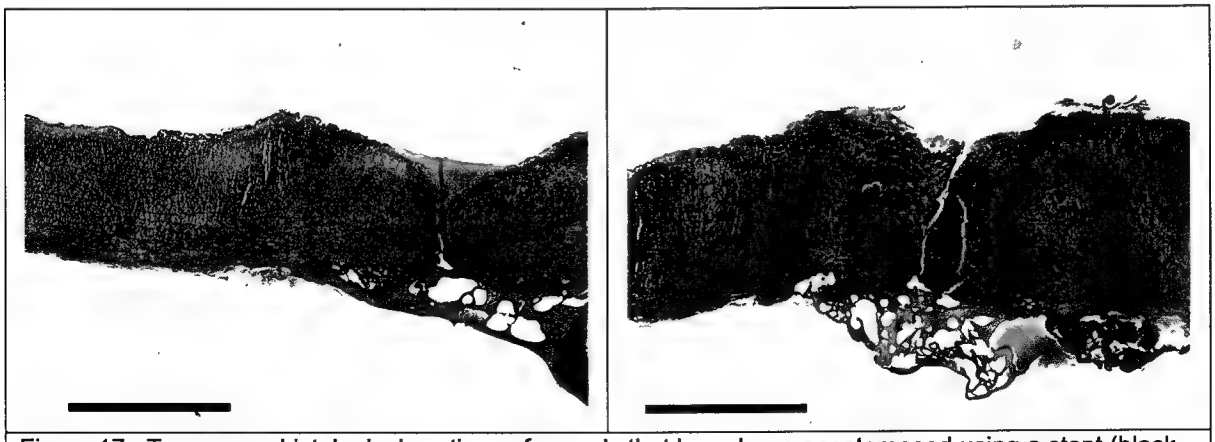


Figure 17. Transverse histological sections of vessels that have been anastomosed using a stent (black bars are 1mm in length). The inner luminal surface is up. The vessel on the left shows that albumin has been coagulated both inside and outside the vessel. This produces an excellent structural bond because

a much larger area is involved in the vessel repair that explains the improved burst strengths of vessels repaired with an albumin stent (compared to repairs without a stent). The image on the right shows that coagulation of the inside albumin stent was not always present.

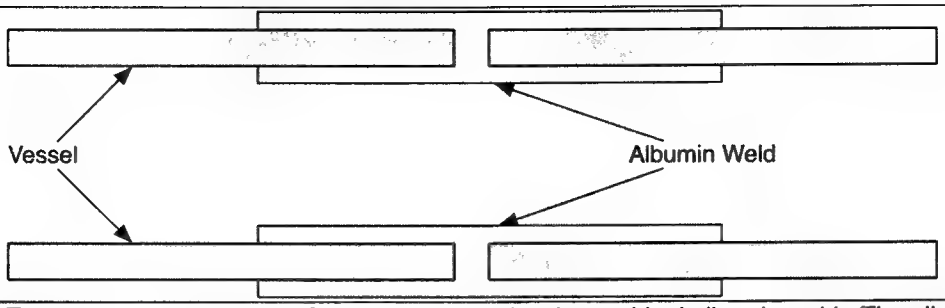


Figure 18. Transverse cross section of repaired vessel showing an ideal albumin weld. The albumin layers on the inside and outside of the vessel provide extensive support for the anastomosis. If there was only albumin between the left and right vessel walls (i.e., none coating the top and bottom) then the repaired vessel would be much weaker.

5. Conclusions

The major result of this work was that the two-wavelength system was effective at joining vessels with strong, water-tight junctions. We found that the albumin stents were useful in the anastomosis process, but it was necessary to closely match the diameter of the stent to that of the vessel; failure to do so made the repair process slower. The albumin stents dissolved quickly in a flowing blood field (about one minute). The albumin stents allowed much better alignment of the vessel edges which, presumeably will lead to improved healing (less scarring and stenosis of the vessel).

The nLIGHT Pearl architecture has proven to be an appropriate architecture for providing 1.9 micron laser light to blood vessel repair. Future versions should have laser die optimized for lower thresholds to allow them to operate cooler, this would allow a more compact package for the laser system.

The aiming beam needs to be done using an LED to avoid the speckle caused by coherent light. In addition using a green light will make the light more distinct from the tissue being illuminated.

The hand piece format appears to be one that is appropriate and if further work is possible it would be further developed for actual operating conditions with essentially the same optical and ergonomic specifications.

6. Recommendations

Given the very promising technique that has been developed for blood vessel repair our team strongly believes that the methods that we have presented could lead to a commercially viable tool that could be applied in both military surgery and in emergency room surgery.

To that end we recommend further development as follows:

1. Add a green LED to the Pearl architecture in a rapid prototype and apply using the laser developed in Phase I to confirm that a green non-coherent light makes a better aiming beam in this application.
2. Fabricate a lower threshold 1.9 micron laser diode to lower the thermal loads required and the laser driver requirements for improved reliability.

3. Develop an operating room ready laser application system with touch screen PC hardware and designed to task circuitry rather than the pieced together system provided in Phase I,
4. Design and fabricate an albumin extrusion machine,
5. Determine optimal stent thickness,
6. Work out the appropriate stent quality assurance metrics,
7. Complete stent sterilization and process verification,
8. Conduct in vitro testing of sterilized, extruded stents,
9. Conduct in vivo testing of final stents.

7. References

[1] R. K. Jain, "Transient temperature distributions in an infinite, perfused medium due to a time-dependent, spherical heat source," J. Biomechanical Engineering, vol. 101, pp. 82–86, 1979.

[2] L. S. Bass and M. R. Treat, "Laser tissue welding: A comprehensive review of current future clinical applications," Lasers Surg. Med., vol. 17, pp. 315–349, 1995.

[3] A. Serure, E. H. Withers, S. Thomsen, and J. Moorris, "Comparison of carbon dioxide laser-assisted microvascular anastomosis and conventional microvascular sutured anastomosis," , Surg Forum, vol. 34, pp. 634–636, 1983.

[4] O. H. Frazier, A. Painvin, J. R. Morris, T. Sharon, and C. R. Neblett, "Laser-assisted microvascular anastomosis: angiographic and anatomopathologic studies on growing microvascular anastomosis: Preliminary report," , Surgery, vol. 97, pp. 585–589, 1985.

[5] W. J. McCarthy, J. LoCicero, R. S. Hartz, and J. S. T. Yao, "Patency of laser-assisted anastomosis in small vessel: One-year follow-up," , Surgery, vol. 102, pp. 319–325, 1987.

[6] B. H. Vale, A. Frenkel, S. Trenka-Benthin, and B. F. Matlaga, "Microsurgical anastomosis of rat carotid arteries with the co2 laser," , Plast Reconstr Surg, vol. 77, pp. 759–766, 1986.

[7] M. R. Quigley, J. E. Bailes, H. C. Kwaan, L. J. Cerullo, and S. Block, "Comparison of myointimal hyperplasia in laser assisted and suture anastomosed arteries," J Vasc Surg, vol. 4, pp. 217–219, 1986.

[8] R. B. Stewart, A. Benbrahim, G. M. LaMuraglia, M. Rosenberg, G. J. LÖltalien, W. M. Abbott, and R. T. V. Kung, "Laser assisted vascular welding with real time temperature control," Lasers Surg Med, vol. 19, pp. 9–16, 1996.

[9] M. M. Judy, J. L. Matthews, R. L. Boriack, et al., "Photochemical cross-linking of proteins with visible-light-absorbing naphthalimides," in Proc. SPIE, vol. 1882, p. 221, 1993.

[10] M. M. Judy, L. Fuh, J. L. Matthews, et al., "Gel electrophoresis studies of photochemical cross-linking of type i collagen with brominated-naphthalimide dyes and visible light," in Proc. SPIE, vol. 2128, p. 506, 1994.

[11] B. P. Chan, I. E. Kochevar, and R. W. Redmond, "Enhancement of porcine skin graft adherence using a light-activated process," J. Surg, vol. 108, p. 77, 2002.

[12] L. Mulroy, J. Kim, I. Wu, et al., "Photochemical keratodesmos for repair of lamellar corneal incisions," Invest. Ophthal. Vis. Sci., vol. 41, p. 3335, 2000.

[13] C. E. Proano, L. Mulroy, E. Jones, et al., "Photochemical keratodesmos for bonding corneal incisions," Invest. Ophthal. Vis. Sci., vol. 45, p. 2177, 2004.

[14] B. P. Chan, C. Amann, A. N. Yaroslavsky, C. Title, D. Smink, B. Zarins, I. E. Kochevar, and R. W. Redmond, "Photochemical repair of achilles tendon rupture in a rat model," J Surg Res, vol. 124, pp. 274–9, 2005.

[15] Y. Kamegaya, W. A. Farinelli, A. V. Vila Echague, H. Akita, J. Gallagher, T. J. Flotte, R. R. Anderson, R. W. Redmond, and I. E. Kochevar, "Evaluation of photochemical tissue bonding for closure of skin incisions and excisions," Lasers Surg Med, vol. 37, pp. 264–70, 2005.

[16] S. Ibusuki, G. J. Halbesma, M. A. Randolph, R. W. Redmond, I. E. Kochevar, and T. J. Gill, "Photochemically cross-linked collagen gels as three-dimensional scaffolds for tissue engineering," Tissue Eng, Feb 22 2007.

[17] M. C. Oz, R. S. Chuck, J. P. Johnson, S. Parangi, L. S. Bass, R. Nowygrod, and M. R. Treat, "Indocyanine green dye-enhanced welding with a diode laser," Vasc Surg, vol. 25, pp. 316–318, 1990.

[18] G. Weng, W. A. Williamson, H. T. Aretz, M. M. Pankratov, and S. M. Shapshay, "Diode laser activation of indocyanine green dye-enhanced albumin for in vitro internal mammary artery anastomosis," Lasers Surg Med, vol. 6, p. 57, 1994.
[19] B. S. Sorg and A. J. Welch, "Laser-tissue soldering with biodegradable polymer films in vitro: film surface morphology and hydration effects," Lasers Surg Med, vol. 28, pp. 297–306, 2001.
[20] A. J. Kirsch, M. I. Miller, D. T. Chang, C. A. Olsson, T. W. Hensle, and J. P. Conner, "Laser tissue soldering in urinary tract reconstruction: first human experience," Urology, vol. 46, pp. 261–266, 1995.
[21] A. J. Kirsch, D. T. Chang, M. L. Kayton, S. K. Libutti, M. R. Treat, and T. W. Hensle, "Laser welding with albumin-based solder: experimental full-tubed skin graft urethroplasty," Lasers Surg Med, vol. 18, pp. 225–230, 1996.
[22] C. S. Cooper, I. P. Schwartz, D. Suh, and A. J. Kirsch, "Optimal solder and power density for diode laser tissue soldering (lts)," Lasers Surg Med, vol. 29, pp. 53–61, 2001.
[23] E. J. Wright, S. M. Schlossberg, and D. P. Poppas, "Evaluation of optimal laser wavelengths and albumin solder concentrations for laser tissue welding," Lasers Surg Med, 1995.
[24] A. Lauto, D. P. Poppas, and G. A. Murrell, "Solubility study of albumin solders for laser tissue-welding," Lasers Surg Med, vol. 23, pp. 258–62, 1998.
[25] D. P. Poppas, E. J. Wright, P. D. Guthrie, L. T. Shlahet, and A. B. Retik, "Human albumin solders for clinical application during laser tissue welding," Lasers Surg Med, vol. 19, pp. 2–8, 1996.
[26] E. N. La Joie, A. D. Barofsky, K. W. Gregory, and S. A. Prahl, "Patch welding with a pulsed diode laser and indocyanine green," Laser Med. Sci., vol. 12, pp. 49–54, 1997.

nLight Photonics
5408 NE 88th Street, Bldg E
Vancouver, WA 98665
www.nlight.net

Point of Contact:
McCal, Dennis
(503) 214-5331
dennis.mccal@nlight.net

Title

Photochemical Tissue Bonding for Military Medical Applications

SBIR Topic Number

AF07-T033

Summary Report Type

Phase I Final

Summation

Joining severed vessels is a recurring problem in trauma and surgery. The basic technology of joining (or anastomosing) vessels using sutures has been available for centuries, but remains a slow and tedious process. Many technologies have been introduced to make vessel suturing water-tight. Any solution to this problem must integrate well with standard medical care. This means that the solution must be safe, effective, acceptable to surgeons, and technologically feasible.

The overall goal of this work was to develop a complete system for micro-anastomosis of vessels. This involved (1) a unique laser system that uses water as the absorbing chromophore, (2) a clinically useful hand piece that is appropriate for microsurgery, (3) a novel albumin stent to support the vessel during anastomosis, and (4) in vitro testing of the device to assess thermal damage, strength, and operative time.

The key values obtained from Phase I of the STTR are that (1) a novel two-wavelength laser system (1.9 and 0.639 micron wavelengths) was produced, (2) an ergonomic handpiece was designed and built, (3) albumin stents were made in a range of sizes and (4) all three components were tested by joining vessels on the benchtop.

The major result of this work was that the two-wavelength system was effective at joining vessels with strong, water-tight junctions. We found that the albumin stents were useful in the anastomosis process, but it was necessary to closely match the diameter of the stent to that of the vessel; failure to do so made the repair process slower. We also found that the red aiming beam (0.639 micron) created a diffuse spot which inhibited the ability of the surgeon to fully exploit the microsurgical precision of the 200 micron spot that the laser produced on the surface of the vessel. Finally, we found that the hand piece design was critical to success of the laser welding process.

We conclude that the two-wavelength system shows exciting promise. We recommend that the aiming beam be changed to a shorter wavelength (green instead of red). We further recommend that albumin stents be produced (under GLP conditions) using molds in a range of sizes from 3-7mm in diameter at 0.5mm increments. With these refinements, the two wavelength anastomosis system can be tested using in vivo pre-clinical experiments.

Anticipated Benefits

Semiconductor lasers at 19xxnm offer particularly strong applications in surgery and dermatology due to the high absorption in water. Many researchers have been interested in 19xxnm lasers due to this unique property, however, historically these applications have been limited by the cost of the lasers that produced these wavelengths – e.g., solid-state and fiber lasers. With recent advances made at nLIGHT,

new efficient and cost effective semiconductor lasers will enable new applications. Lasers have been used in a wide range of applications in dermatology for many years. The largest single market is in laser hair removal (over $60 million in sales per year) however, the high water absorption of 19xx nm lasers, offer many applications including treatments for pigmented lesions, skin resurfacing, acne, and rosacea. A surgical application similar to the subject of this STTR is in laparoscopy for sealing vessels accidentally opened during surgery. Adding albumin to the surface of the leaking vessel and coagulating the albumin allows bleeding to be readily controlled. In military the availability of of fiber coupling results in high brightness pump sources suitable for the end pumping of Ho:YAG solid state lasers. The Ho:YAG solid state lasers may be used directly in infrared countermeasures or to pump Optical Parametric Oscillators for the creation of high-power sources with emission wavelengths of 3-4 microns or greater. Similarly Tm/Ho direct pumping at 19xxnm wavelengths would lase efficiently around 2 micron with applications in LARDAR and DE systems.
